

Voluntary running does not reduce neuroinflammation or improve non-cognitive behavior in the 5xFAD mouse model of Alzheimer's disease

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Supplementary Data

Supplementary Table 1- Body weights

	Sedentary (Mean±SD)	Running (Mean±SD)	T-test (unpaired) Sedentary vs. Exercised
Start Weight (g)	16.8±1.1	16.1±1.3	P=0.14
Final Weight (g)	23.4±1.3	22.6±3.0	P=0.35
Weight gain (%)	40±11	40±16	P=0.98

Supplementary Table 2- Corticosterone levels in feces (ELISA)

	Sedentary Median (IQR)	Running Median (IQR)	Mann Whitney U-test Sedentary vs. Running
Baseline levels (pg/ml)	2617 (1699-4455)	2167 (1644-4053)	P=0.66
After 19 weeks (pg/ml)	1523 (1331-2205)	1506 (1237-1722)	P=0.85
Wilcoxon test Baseline vs. After 19 weeks	P=0.001	P=0.02	

Supplementary Table 3- distance traveled in EPM and OF

	Sedentary Median (IQR)	Running Median (IQR)	Mann Whitney U-test Sedentary vs. Running
Distance moved Elevated plus maze	1048 (741-1357)	1278 (964-1616)	P=0.23
Distance moved Open field	4569 (3115-5561)	4017 (3746-5568)	P=0.93

Supplementary Table 4- Sucrose Preference

	Sedentary Median (IQR)	Running Median (IQR)	Mann Whitney U-test P-value
Sucrose Preference (%)	79.6 (76.4-86.1)	79.7 (73.2-88.4)	1.0

Supplementary Table 5- A β levels (ELISA)

	Different Aβ species	Sedentary concentration (ng A β /mg protein) Median (IQR)	Running concentration (ng A β /mg protein) Median (IQR)	Mann Whitney U- test p-values prior to Bonferroni correction
Insoluble fraction in hippocampus	A β -38	164.5 (131.5- 322.4)	255.3 (147.8- 303.4)	0.57
	A β -40	1048 (712.4- 1286)	1194 (989.7-1526)	0.35
	A β -42	8007 (5909- 10173)	9612 (4285- 11546)	0.78
CSF (n=7+7)	A β -38	0.59 (0.54-0.88)	0.44 (0.19-0.54)	0.21
	A β -40	3.87 (2.17-4.92)	2.28 (0.76-3.23)	0.32
	A β -42	1.81 (1.18-2.46)	1.25 (0.35-1.68)	0.32

Supplementary Table 6- Iba1 and gal-3 in hippocampus (immunohistochemistry)

	Sedentary Median (IQR) Fold to actin %	Running Median (IQR) Fold to actin %	Mann Whitney U-test P-value
Galectin-3 (n=6+6)	116 (84-125)	114 (102-127)	0.94
NLRP3 (n=3+4)	22 (18-33)	17 (16-20)	0.63

Supplementary Table 7- Cytokine levels (ELISA)

	Cytokine	Sedentary Mean concentration (pg/ml) \pm SD	Running Mean concentration (pg/ml) \pm SD	T-test P-values prior to Bonferroni correction
Hippocampus	IL-1 β	1.29 \pm 0.7	1.34 \pm 0.8	0.85
	IL-2	0.022 \pm 0.01	0.029 \pm 0.02	0.26
	IL-4	0.045 \pm 0.02	0.052 \pm 0.04	0.58
	IL-5	0.008 \pm 0.003	0.010 \pm 0.005	0.26
	IL-6	0.45 \pm 0.3	0.68 \pm 0.7	0.27
	IL-10	0.15 \pm 0.09	0.15 \pm 0.07	0.99
	IL-12p70	1.57 \pm 0.8	1.78 \pm 0.9	0.52
	IFN γ	Below detection	Below detection	
	TNF α	0.067 \pm 0.02	0.082 \pm 0.06	0.40
KC/GRO	2.75 \pm 1.9	2.94 \pm 2.6	0.82	
Serum	IL-1 β	0.27 \pm 0.2	0.20 \pm 0.2	0.37
	IL-2	0.12 \pm 0.1	0.13 \pm 0.1	0.84
	IL-4	Below detection	Below detection	
	IL-5	1.48 \pm 1.3	1.68 \pm 1.0	0.64
	IL-6	3.25 \pm 2.6	3.59 \pm 4.2	0.80
	IL-10	7.14 \pm 3.9	6.14 \pm 3.6	0.48
	IL-12p70	Below detection	Below detection	
	IFN γ	0.33 \pm 0.1	0.20 \pm 0.1	0.06
	TNF α	3.66 \pm 1.0	4.17 \pm 1.8	0.38
KC/GRO	34.14 \pm 29.8	23.52 \pm 10.2	0.22	

Supplementary Figure S1- Distance in running wheels

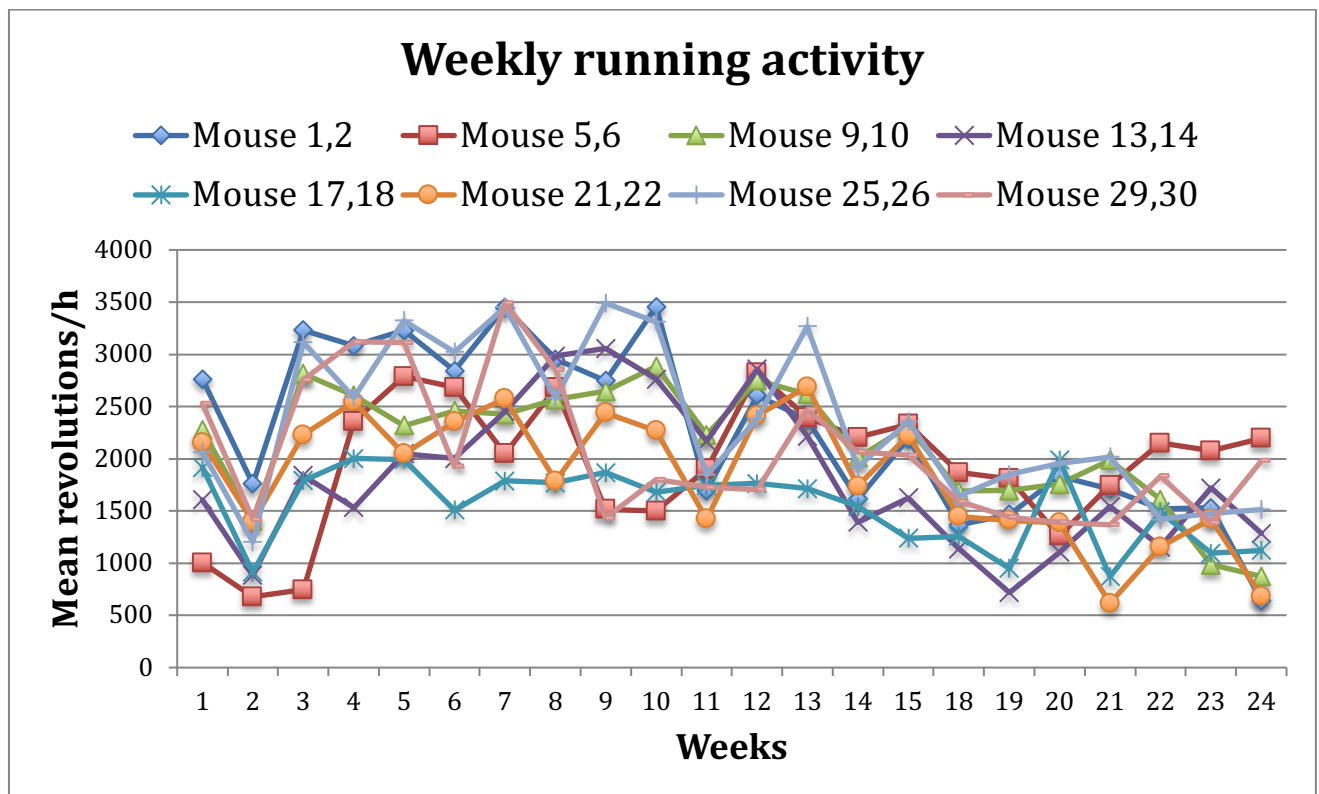


Figure S1. Running activity displayed as mean revolution per hour for each week and each couple sharing a running wheel in their home cage. Each revolution corresponds to a running distance of around 42 cm. ANOVA repeated measurements showed a change in the amount of running over time ($p < 0.001$) and a paired T-test comparing the running during the first week with that of the last week of intervention revealed a trend towards decreased running over time (means \pm SD were 2039 ± 549 revolutions/h during the first week compared to 1288 ± 578 revolutions/h during the last week, paired T-test, $p = 0.07$).

Supplementary Methods

Sucrose preference test

To assess anhedonic behavior, a Sucrose preference test was performed during the night before sacrifice. Mice were introduced to a sucrose solution in their home cages one night before the test. A bottle containing 2% sucrose solution was put in the place where the regular bottle with tap water used to be during the night. The regular bottle with tap water was placed in the other corner of the cage, allowing the mice to choose. The day before the test, mice were deprived from drinking five hours prior to the test. Later, mice were individually caged with access to nesting material, food pellet, as well as two bottles, one tap water and one sucrose solution as described

before ¹. Bottles were weighed before and after the test and the volume consumed was calculated. A sucrose preference index was calculated using the following formula:

Sucrose preference index = weight of consumed sucrose / total weight consumed of both solutions

Multiplex ELISA

Cytokine ELISA

The concentrations of different cytokines in serum as well as in the pooled first and second fraction of homogenized hippocampus (25 µl/sample) were measured with the MSD Mouse Proinflammatory V-Plex Plus Kit (IFN γ , IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p70, CXCL1, TNF α ; K15012C, Mesoscale) using a QuickPlex SQ120 (Mesoscale Discovery, Rockville, USA) Plate Reader according to the manufacturer's instructions. The recorded data was analyzed using MSD Discovery Workbench software. For the brain homogenate samples, the cytokine concentrations were normalized to the total protein concentrations measured in the BCA or Bradford assay.

A β ELISA

The concentration of different A β species in the insoluble fraction of homogenized hippocampus as well as in the CSF were measured with the MSD MULTI-SPOT Human (4G8) A β Triplex Assay (A β 38, A β 40 and A β 42; K15199G-1, Mesoscale) using QuickPlex SQ120 (Mesoscale Discovery, Rockville, USA) Plate Reader according to the manufacturer's instructions. The recorded data was analyzed using MSD Discovery Workbench software. For the brain homogenate samples, A β concentrations were normalized to total protein concentrations measured in the BCA or Bradford assay.

Fecal corticosterone levels

Fecal samples were collected from the Open field arena after conducting the Open field test in order to measure the stress levels of the mice. The feces were stored at -80°C until use. Corticosterone was then extracted and analyzed with a corticosterone ELISA kit (Enzo Life Sciences) described by Touma et al.² except that feces was homogenized in 1 ml of 80% Methanol per 100 mg sample, as we have done before³.

References

- 1 Bay-Richter, C. *et al.* Behavioural and neurobiological consequences of macrophage migration inhibitory factor gene deletion in mice. *J Neuroinflammation* **12**, 163, doi:10.1186/s12974-015-0387-4 (2015).
- 2 Touma, C., Sachser, N., Mostl, E. & Palme, R. Effects of sex and time of day on metabolism and excretion of corticosterone in urine and feces of mice. *Gen Comp Endocrinol* **130**, 267-278, doi:S0016648002006202 [pii] (2003).
- 3 Svensson, M. *et al.* Forced treadmill exercise can induce stress and increase neuronal damage in a mouse model of global cerebral ischemia. *Neurobiol Stress* **5**, 8-18, doi:10.1016/j.ynstr.2016.09.002 (2016).